

P-270 - ESTABLISHMENT OF GALACTOSE UTILIZATION IN *SACCHAROMYCES CEREVISIAE* PE-2 THROUGH EXPRESSION OF THE CEN.PK113-5D GAL2 GALACTOSE PERMEASE

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Background

Saccharomyces cerevisiae PE-2 is one of the most robust yeast chassis for use in second-generation bioprocesses [1-2]. Unfortunately, we noticed that it is incapable of utilizing galactose, which is abundant in diverse agro-industrial derived substrates. In this study we analysed the galactose utilization pathway (Leloir pathway) of this strain and identified the Gal2 galactose permease as the limiting step.

Method

The putative amino acid (aa) sequences of the *S. cerevisiae* JAY291 (haploid derivative of PE-2) proteins involved in galactose utilization (Gal2, Gal1, Gal7, Gal10, Gal5, Gal4, Gal80 and Gal3) were aligned (Clustal Omega) with the corresponding proteins of other industrial and laboratorial strains, revealing several point mutations in the Gal2 permease. PE-2 was then transformed with a 2 micron plasmid containing the CEN.PK113-5D *GAL2* under the regulation of the *TDH3* promoter and *PGI1* terminator, and the resulting transformants were physiologically characterized in liquid YP containing 2% galactose plus 150 µg/mL G418.

Results & Conclusions

Homology-based analysis of the *S. cerevisiae* PE-2 Leloir pathway allowed the identification of 12 aa substitutions in the Gal2 sequence that are not conserved across other industrial and laboratorial strains. Three of these point mutations were found in the transmembrane domain 7 (TM7), a region important for substrate recognition [3]. Among these, the most significant includes the substitution F336L, as the loss of aromatic aa in TM7 is reported to be critical for galactose transport activity [3]. These results suggested that the galactose permease of *S. cerevisiae* PE-2 might lack galactose transport activity, which was further supported by the fact that expression of the CEN.PK113-5D *GAL2* in PE-2 established its galactose utilization capacity. In fact, with this modification PE-2 was faster than CEN.PK113-5D in consuming 2% galactose (12h vs 14h, respectively). The high galactose utilization efficiency of this newly constructed PE-2 strain opens new perspectives and opportunities for the valorisation of galactose-containing second-generation substrates.

References & Acknowledgments

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